Effect of High-Molecular-Weight Glutenin Subunit Allelic Composition on Wheat Flour Tortilla Quality

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ABSTRACT

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Wheat cultivars possessing quality attributes needed to produce optimum quality tortillas have not been identified. This study investigated the effect of variations in high-molecular-weight glutenin subunits encoded at the *Glu-1* loci (*Glu-A1, Glu-B1,* and *Glu-D1*) on dough properties and tortilla quality. Flour protein profiles, dough texture, and tortilla physical quality attributes were evaluated. Deletion at *Glu-D1* resulted in reduced insoluble polymeric protein content of flour, reduced dough compression force, and large dough extensibility. These properties produced very large tortillas (181 mm diameter) compared with a control made with commercial tortilla wheat flour (161 mm). Presence of a 7 + 9 allelic pair at *Glu-B1* increased dough strength (largest compression force, reduced extensibility, and small-diameter tortillas). Deletion at *Glu-A1* produced large tortillas (173 mm) but with unacceptable flexibility during storage (score <3.0 at day 16). In general, presence of 2* at *Glu-A1*, in combination with 5 + 10 at *Glu-D1*, produced small-diameter tortillas that required large force to rupture (tough texture). Presence of 2 + 12 alleles instead of 5 + 10 at *Glu-D1* produced tortillas with a good compromise between diameter (>165 mm) and flexibility during storage (>3.0 at day 16). These allele combinations, along with deletion at *Glu-D1*, show promise for tortilla wheat development.

The growing popularity of tortillas is attributed to their convenience as wraps that suit an on-the-go lifestyle. In the United States, consumers prefer refined wheat flour tortillas that are flexible, opaque, large in diameter, and have a long shelf life (Bello et al 1991; Cepeda et al 2000). However, no wheat cultivars have been identified that possess the intrinsic quality attributes needed for the production of optimum quality tortillas. Currently, the tortilla industry uses bread wheat flour and chemical ingredients, for example, reducing agents, to achieve the required functionality for tortilla production. Without modification, hard winter wheat cultivars developed for bread produce poor quality tortillas that are small in diameter and chewy or tough (Serna-Saldivar et al 2004), because protein functionality requirements for wheat flour tortillas differ from those required for good quality bread. The desirable protein network (gluten) for good quality tortilla production is extensible and mellow, whereas bread dough requires a strong, resilient gluten network to retain air bubbles during fermentation.

Dough extensibility is essential for the production of large-diameter tortillas (Pascut et al 2004). Both diameter and shelf stability (flexibility over time) are believed to be controlled more by wheat glutenin and gliadin over any other endosperm subfractions such as globulin, albumin, starch, or lipids (Waniska et al 2004). Hence, there is opportunity to select for specific glutenin and gliadin composition in wheat cultivars to produce wheat with the unique requirements for tortilla production. End-use quality variations are governed by the glutenin/gliadin ratio and by molecular weight distribution in glutenins, which can be genetically determined (Cinco-Moroyoqui and MacRitchie 2008). Molecular weight distribution is dependent on variations in the high-molecular-weight (HMW) glutenin allelic composition (Gupta and MacRitchie 1994), availability of chain terminators (Masci et al 1998), and the ratio of low-molecular-weight (LMW) to HMW glutenins (Gupta et al 1993). In hexaploid wheat, the HMW glutenin subunits (HMW-GS) are encoded at the *Glu-1* loci (*Glu-A1, Glu-B1,* and *Glu-D1*) located on the long arms of the group 1 chromosomes (1A, 1B, and 1D) (Payne and Lawrence 1983). Three complex *Gli-1/Glu*-3 loci (*Gli-A1/Glu-A3, Gli-B1/Glu-B3,* and *Gli-D1/Glu-D3*) on the short arms of chromosomes 1A, 1B, and 1D code for ω- and γ-gliadins and LMW glutenins, respectively (Payne et al 1987). These loci contain allelic variations.

Each HMW-GS locus contains two tightly linked genes that encode the x- and y-type subunits (for example, 1Ax, 1Ay, 1Bx, 1By, 1Dx, and 1Dy). However, because of gene silencing, HMW-GS 1Ax and 1By are not expressed in some cultivars, and subunit 1Ay is always silenced, resulting in expression of three-to-five HMW-GS (Payne et al 1987).

Each of these subunits affects dough quality. The allelic pairs encoded on the *Glu-D1* locus (5x + 10y, 2x + 12y), followed by the single subunit at the *Glu-A1* (1, 2*, null) and those at *Glu-B1* (20x + 20y, 7x + 9y, 17x + 18y), are the principal components of quality (Payne et al 1987). The most significant of these alleles occur at *Glu-D1*, where HMW-GS can occur as allelic pairs of genes encoding HMW-GS designated as 5 + 10 or 2 + 12 (MacRitchie and Lafiandra 2001). Mondal et al (2008) reported that wheat varieties possessing HMW-GS 17 + 18 at the *Glu-B1* loci with deletions in the *Glu-A1* and *Glu-D1* loci gave large-diameter tortillas but with some loss in flexibility. However, there is no information about the effect of 2 + 12 compared with 5 + 10 at *Glu-D1* on tortilla quality or about the role variations in allelic composition at *Glu-A1* and *Glu-B1* may play in increasing the extensibility of dough for large-diameter flour tortillas.

There is a need to increase understanding of the roles different HMW-GS alleles play toward ideal tortilla quality in an effort to utilize this information in developing wheat varieties for tortilla production. The goal of the study was to evaluate the tortilla-making properties of wheat lines possessing variations in HMW glutenin alleles at homologous loci on A, B, and D genomes.

MATERIALS AND METHODS

Wheat Samples

Fifteen wheat lines with variations in HMW-GS composition (Table I) were planted in two fields (Texas Agricultural Experiment Station at McGregor, Texas, in 2008 and 2009 and the Texas AgriLife Research Station at College Station, Texas, in 2009),

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thus providing three "environments." The wheat was harvested, milled, and processed into tortillas. Lines with similar allelic composition were grouped together (Table I).

Evaluation of Grain and Flour Properties

Kernel weight and hardness scores were obtained from an average of 300 kernels/sample through the single-kernel characterization system (SKCS, Perten Instruments, Springfield, IL). Wheat samples were tempered to 15% moisture and milled according to AACC International Approved Method 26-50.01 (AACCI 2010) with a Quadrumat Jr. experimental mill (Brabender, South Hackensack, NJ). Straight-grade flour produced by blending break and reduction flours was used for all quality analyses and evaluations; mean flour yield was 66.0% (range 59.3–72.6%). Flour protein content was determined by near-infrared reflectance spectroscopy following AACCI Approved Method 39-11.01 (2010).

Flour quality parameters including mixing time, water absorption, and mixing tolerance were measured with a mixograph (National Manufacturing, Lincoln, NE) following AACCI Approved Method 54-40.02 (2010). Mixograph peak times ranged averaged 3 min (2–4 min), whereas dough water absorption averaged 62.9% (61–64%). These data were used to determine optimum dough mixing conditions for each sample.

Protein Analysis

HMW-GS Identification with Lab-on-a-Chip. Lab-on-a-chip electrophoresis was performed to determine the HMW-GS composition of flour samples using the Agilent 2100 bioanalyzer with a Protein 230 chip kit (Agilent Technologies, Palo Alto, CA), as described previously (Mondal et al 2008). Wheat samples of well-known subunit composition were selected as controls to compare the apparent molecular sizes to facilitate the identification and comparison of HMW-GS. The standards used were Chinese Spring (null, 7 + 8, 2 + 12), Karl-92 (1, 7 + 8, 5 + 10), and Jagger (1, 17 + 18, 5 + 10).

Polymeric Protein Analysis. The percentage of insoluble polymeric proteins (IPP) was determined as described by Bean et al (1998). A 10 mg flour sample was suspended in 1.0 mL of 0.5% (w/v) SDS buffer. The suspension was then stirred for 5 min at 20,000 × *g* and centrifuged for 20 min at 15,900 × *g* to obtain a supernatant (extractable polymeric protein [EPP]). The residue was then sonicated for 30 sec in 0.5% (w/v) SDS buffer (1.0 mL) to solubilize the remaining protein (unextractable polymeric protein [UPP]). Both the extracts were filtered through 0.45 μm filters. The percentages of EPP and UPP were calculated as previously described (Bean et al 1998).

Size-Exclusion HPLC. Flour protein compositions were determined quantitatively following the size-exclusion HPLC extraction procedure described by Gupta et al (1993). Briefly, a 10 mg sample was used to extract total polymeric protein (TPP), EPP, and UPP fractions. TPP was extracted with 2% SDS buffer for 5 min by vortexing, sonicated for 15 sec (6W output), and centrifuged (model 1514, Eppendorf, Westbury, NY) for 20 min at 13,400 × *g*. A similar procedure was followed for EPP (with the exception of sonication), and the residue was used for the UPP fraction. UPP was extracted for 10 min and centrifuged for 20 min (13,400 × *g*) after sonication for 25 sec. Protein samples were stabilized before analysis by heating the samples in a water bath at 85°C for 5 min and cooled by storage in ice. Stabilized protein extracts were injected (20 μL) onto a Phenomenex Biosep-SEC-S4000 (300 × 7.8 mm) size-exclusion column (Phenomenex, Torrance, CA) connected to an Agilent 1100 HPLC system. Data analysis was performed with the Agilent ChemStation software program. A variable wavelength detector set to 214 nm was used. The column temperature was 40°C. Solvent was 50% acetonitrile in water v/v, acidified with 0.05% trifluoroacetic acid. A flow rate of 1.0 mL/min was employed, with a total runtime of 28 min.

TABLE I
High-Molecular-Weight Glutenin Allele Composition of Wheat Lines

Group	Entry	Wheat Lines	*Glu-A1*	*Glu-B1*	*Glu-D1*
1	1	Gabo	2*	17 + 18	2 + 12
2	2	Ogallala	2*	20x + 20y	5 + 10
2	11	TX04CS00237	2*	20x + 20y	5 + 10
5	5	TX04CS00233	1	17 + 18	5 + 10
5	9	TX04CS00235	1	17 + 18	5 + 10
5	14	TX04CS00240	1	17 + 18	5 + 10
5	18	TX04CS00245	1	17 + 18	5 + 10
6	6	TX04CS00229	2*	17 + 18	5 + 10
7	7	TX04CS00230	…	17 + 18	5 + 10
8	8	TX04CS00232	2*	17	2 + 12
10	10	TX04CS00236	2*	7 + 9	2 + 12
10	19	TX04CS00249	2*	7 + 9	2 + 12
13	13	TX04CS00239	1,2*	17 + 18	2 + 12
15	15	TX04CS00241	1,2*	7 + 9	5 + 10
16	16	TX04CS00231	2*	17	5
20	20	Control	Unknown	Unknown	Unknown

Tortilla Formulation and Dough Properties

Tortilla formulation was as described by Alviola and Awika (2010); amount of water for dough preparation was based on farinograph or mixograph water absorption of each sample.

Dough compression force was measured as described by Bejosano et al (2005) and Barros (2009); two dough balls of approximately equal weight (45 g) were subjected to 70% compression with a 10 cm diameter probe on a texture analyzer (TA.XT2i, Texture Technologies, Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, U.K.). Maximum dough compression force was recorded and averaged for each of the treatments.

Dough extensibility was measured as reported by Smewing (1995) with the Kieffer dough and gluten extensibility rig attached to a texture analyzer. After resting the dough balls for 10 min in the proofing chamber (32°C, 70% rh), 20 g from one dough ball was taken and rolled into a cylindrical shape, placed into the grooved mold, rested for 40 min at room temperature, and evaluated. Dough extensibility and resistance to extension were determined.

Tortilla Processing

Tortillas were prepared according to the standard hot-press method (Bello et al 1991). Dough balls were hot-pressed for 1.4 sec at 1,150 psi and 205°C, baked for 30 sec at 194–199°C on a three-tier gas-fired oven (model 0P01004-02, Lawrence Equipment, El Monte, CA), and then cooled for 1.5 min on a three-tier conveyor (Superior Food Machinery, Pico Rivera, CA). Immediately after cooling, each tortilla was placed on a metal table for 2 min and then packaged in polyethylene bags and stored at 22°C until analyzed.

Evaluation of Tortilla Physical Properties

Evaluation of Fresh Tortillas. Ten tortillas were randomly selected, weighed, and measured for diameter, height, and opacity as described by Friend et al (1995). Tortilla moisture was determined following a two-stage procedure in a hot-air oven (Approved Method 44-15.02, AACCI 2010). Tortilla opacity (%) was evaluated subjectively on a 100 point scale for 10 tortillas from each wheat line and control. A highly opaque tortilla was given a 100% rating, and completely translucent tortillas were rated as 0%.

Tortilla color values L^* (whiteness), a^* (red-green), and b^* (yellow-blue) were measured at two points on each side of two randomly selected tortillas from each treatment with a Minolta color meter (Chroma Meter CR-310, Minolta, Tokyo, Japan).

Rollability and Flexibility. Tortilla shelf stability was evaluated subjectively at days 4, 8, 12, and 16 of storage by a rollability/flexibility test, as described by Friend et al (1995). Each of 10 random tortillas was wrapped around a 1.0 cm diameter wooden dowel and rated from 1 (breaks easily, cannot be rolled) to 5 (no cracks, very flexible).

Tortilla Texture—Two-Dimensional Extensibility. Tortilla textural changes during storage were measured at days 0, 4, 8, 12, and 16 with the two-dimensional extensibility tests of Bejosano et al (2005) on a TA.XT2i texture analyzer. The extensibility test was conducted using the return to start option, at a trigger force of 0.05 N. Pre- and posttest speed was 10.0 mm/sec. Test speed was 1.0 mm/sec. The modulus of deformation (N/mm), force (N), distance (mm), and work to rupture (N·mm) were measured.

Experimental Design and Data Analysis

A randomized complete block design with growth environment as the block and allelic composition as the treatment was used. Statistical analysis was done with SAS version 9.2 (SAS Institute, Cary, NC). Analysis of variance and least significant difference tests were performed at the $\alpha = 0.05$ significance level to determine differences among the samples and treatments. Analysis of samples within each environment was conducted on two separate occasions. Treatment × environment interactions were not significant.

RESULTS AND DISCUSSION

Flour Protein Properties

The flour protein content for the samples averaged for the three growing environments ranged between 12.2 and 13.7% (dry basis) and was within normal variability expected for genetically related wheat lines (Table II). The standard deviations were generally modest (below 5%), indicating that growing environment had minimal effect on protein content. IPP values ranged between 33.4 and 45.8% (based on protein content) and were within the expected range (Park et al 2006). In general, there was no correlation observed among protein content, IPP, GS ratios, and glutelin/gliadin ratio (Table II). Interestingly, however, sample group 16, which possessed HMW-GS 1Ax2*, 1Bx17, and 1Dx5, not only had the highest protein content (13.7%) and highest glutenin/gliadin ratio but also had the lowest IPP content and its HMW-GS/LMW-GS ratio was among the lowest (Table II). The low IPP content agrees with findings by Mondal et al (2008) that deletion at *Glu-D1* results in decreased IPP (%). On the other hand, groups with the highest IPP contents (1, 5, 6, and 15) had 5 + 10 at *Glu-D1*, with the exception of group 1, which had 2 + 12. Payne et al (1987) reported that 5 + 10 at the *Glu-D1* locus contributed to dough strength, whereas IPP content is one of the major determinants of dough strength and showed higher correlations than protein content to bread loaf volume, bake mix time, and mixing tolerance (Bean et al 1998; Park et al 2006). A strong negative correlation has been shown between IPP and tortilla properties (Pierucci et al 2009; Barros et al 2010).

However, it is important to note that wheat flours with identical IPP composition can produce dough with dramatically different mixing properties, owing to other factors that determine gluten network formation, for example, number of cysteine residues in the gluten polymer (Shewry et al 1992).

Dough Properties

Mean dough compression force ranged from 82 to 127 N, with sample groups 2, 8, and 16 having significantly lower compression force (82–94 N) than the control (110 N) (Table III). Compression force is a measure of dough stiffness and indirectly of

TABLE III
Effects of Different High-Molecular-Weight Glutenin Allele Composition on the Objective Dough Properties[a]

Group	Entry	Compression Force (N)	Resistance to Extension (N)	Extensibility (mm)
1	1	103cd (72–136)	0.31e (0.23–0.38)	103ab (80–136)
2	2,11	82f (67–95)	0.21f (0.12–0.35)	107a (80–120)
5	5,9,14,18	103cd (60–122)	0.38cd (0.12–0.73)	72d (30–104)
6	6	98cde (84–109)	0.33de (0.31–0.34)	93c (85–108)
7	7	108cd (96–126)	0.20f (0.8–0.22)	92c (84–99)
8	8	94def (82–103)	0.20f (0.13–0.19)	105ab (77–125)
10	10,19	127a (88–151)	0.49b (0.17–0.81)	72d (33–112)
13	13	106cd (101–114)	0.24f (0.22–0.36)	98bc (89–105)
15	15	125a (115–140)	0.41c (0.37–0.47)	74d (56–92)
16	16	87ef (75–95)	…	>150*
20	Control	110bc (90–126)	0.71a (0.60–0.85)	28e (24–41)
LSD		15	0.05	8.4

[a] Average from two trials of lines planted in three locations (range of values in parentheses). Values followed by the same letter in the same column are not significantly different ($P < 0.05$). LSD = least significant difference ($P < 0.05$), and * indicates extensibility outside of the range measurable with the TA.XT2i texture analyzer.

TABLE II
Effects of Different High-Molecular-Weight (HMW) Glutenin Allele Composition on the Flour Protein Profile[a]

Group	Entries[b]	Ratios: Glutenin/Gliadin	Ratios: HMW-GS/LMW-GS	IPP[c] (%)	Protein (%)
1	1	1.9 ± 0.2abc	0.43 ± 0.0ab	45.8 ± 2a	13.3 ± 0.2ab
2	2,11	2.0 ± 0.2ab	0.31 ± 0.1c	39.6 ± 6a–d	13.2 ± 0.5ab
5	5,9,14,18	1.7 ± 0.2bc	0.40 ± 0.1abc	44.2 ± 6ab	12.8 ± 0.5bcd
6	6	1.9 ± 0.1abc	0.40 ± 0.1abc	43.9 ± 3ab	13.1 ± 0.4abc
7	7	1.7 ± 0.3bc	0.37 ± 0.1abc	34.9 ± 5cd	13.1 ± 0.4abc
8	8	1.9 ± 0.2abc	0.46 ± 0.1a	38.5 ± 3bcd	13.3 ± 0.5ab
10	10,19	1.7 ± 0.2c	0.30 ± 0.0c	41.3 ± 5abc	12.2 ± 0.5d
13	13	1.8 ± 0.1abc	0.33 ± 0.1bc	40.1 ± 4a–d	13.3 ± 0.5ab
15	15	1.9 ± 0.0abc	0.43 ± 0.0ab	45.2 ± 2ab	12.5 ± 0.6cd
16	16	2.0 ± 0.1a	0.31 ± 0.0c	33.4 ± 4d	13.7 ± 0.4a
20					12.5 ± 0.1cd
LSD		0.3	0.1	7.3	0.7

[a] Average from two trials of lines planted in three locations. Values followed by the same letter in the same column are not significantly different ($P < 0.05$). LSD = least significant difference ($P < 0.05$); LMW = low molecular weight; GS = glutenin subunit; and IPP = insoluble polymeric protein.
[b] Wheat lines with similar HMW glutenin allele composition.
[c] As a percentage of protein.

dough strength. These three sample groups also had the lowest resistance to extension (indicating lower dough strength or elasticity), as well as the highest dough extensibility (105 to >150 mm). In general, the three groups that had the lowest compression force and resistance to extension lacked 17 + 18 or 7 + 9 on *Glu-B1*; this observation suggests that these alleles have a significant impact on dough properties. For sample group 16, extensibility was so large that it exceeded the 150 mm limit of the texture analyzer. This large extensibility was partly expected, based on the protein profile for this sample, that is, low IPP content and HMW-GS/LMW-GS ratio (Table II). In addition, the absence of allele pair 10y from *Glu-D1* likely contributed to the unusually extensible and soft dough for this sample group. Because dough extensibility is positively correlated with tortilla diameter (Barros et al 2010), this sample group is likely to produce large-diameter tortillas, a desirable attribute. Dough that requires high force to extend (resistance to extension) is very elastic and shrinks back after pressing, thereby producing small-diameter tortillas that also tend to be chewy (Wang and Flores 1999).

On the other hand, sample groups 10 and 15 had the highest mean dough compression force (125–127 N) and resistance to extension (0.41–0.49 N) and among the lowest extensibility scores (72–74 mm; Table III). Sample groups 10 and 15 were the only lines with a 7 + 9 *Glu-B1* locus (Table I), which may partly explain their dough strength; however, no published reports are available on the effect of this HMW-GS pair on dough properties. The observation needs further investigation. The strong dough attributes of these lines are likely to produce undesirable, small-diameter tortillas because of shrink-back of the highly elastic dough after pressing.

In general, presence of 17 + 18 on *Glu-B1* resulted in dough with intermediate compression and extension properties, which may provide the delicate compromise between extensibility and flexibility over time important for tortilla quality. It is important to note that all the lines tested had significantly lower resistance to extension and higher extensibility than did the commercial tortilla flour used as the control, which suggests that they may be more suitable for tortillas than the available commercial tortilla flour.

Tortilla Properties

Opacity and L Value.* Tortilla opacity is an important quality attribute, especially in the United States, where opaque tortillas are preferred because tortillas that appear translucent are perceived as greasy (Waniska et al 2004). Opacity depends on the extent of retention of gas bubbles produced by leavening agents (Alviola and Awika 2010). All tested lines produced tortillas that were largely opaque and comparable to or better than the control in opacity score (Table IV). Likewise, *L** values, which generally correlate with the subjective opacity scores (Alviola and Awika 2010), were high and ranged between 80.5 and 83.0. These values were comparable to the control (81.5) and similar to previous reports (Alviola et al 2010).

Tortilla Diameter. Tortilla diameter is one of the key quality attributes because a tortilla is primarily meant to function as a wrap. Large-diameter tortillas are preferred. The diameter for the tortilla lines averaged between 163 and 181 mm, compared with that of the control at 161 mm (Fig. 1). Sample groups 2, 7, 8, 13, and 16 had significantly larger diameter than the control. This observation generally agreed with the previously discussed dough properties data, which indicated that doughs from groups 2, 8, and 16 had the lowest compression force and resistance to extension, while also being the most extensible (Table III). Importantly, sample group 16, which produced the most extensible dough, also produced the largest diameter tortillas (mean = 180.7 mm; range 172–185 mm). This diameter was 12% larger than the control tortilla, which translates to 25% larger surface area. Thus, the combination of subunits 17 on *Glu-B1* and 5 on *Glu-D1* seemed to be a very promising allelic composition for tortilla wheat.

Even though sample groups 7 and 13 produced doughs that were intermediate in textural attributes (Table III), they produced tortillas that had relatively large diameters compared with the control, and they were comparable to group 2 and 8 tortillas (Fig. 1). A possible explanation for larger diameter tortillas for group 7 is the deletion of the allele at the *Glu-A1* locus, which somewhat counteracted the strengthening effect of 17 + 18 and 5 + 10 allelic combinations on the *Glu-B1* and *Glu-D1* loci, respectively, thus reducing the ability of the dough for this group to shrink back after pressing. MacRitchie and Lafiandra

TABLE IV
Effects of High-Molecular-Weight Glutenin Allele Composition on Tortilla Physical Properties[a]

				Tortilla Flexibility Score		
Group	Entry	Opacity	*L** Value	Day 8	Day 12	Day 16
1	1	80a–d	80.5d	4.9a	4.3ab	3.8abc
		(72–85)	(80–81)	(4.9–5.0)	(4.1–4.8)	(3.4–4.1)
2	2,11	85abc	83.0a	4.4c	3.6d	2.8ef
		(74–99)	(81–85)	(2.6–5.0)	(2.4–4.6)	(1.3–3.9)
5	5,9,14,18	82a–d	81.9abc	4.4bc	4.0a–d	3.7a–d
		(69–95)	(80–84)	(2.3–5.0)	(2.8–5.0)	(2.1–5.0)
6	6	75d	82.3abc	4.6abc	4.4ab	3.9ab
		(68–84)	(80–84)	(4.5–5.0)	(3.7–5.0)	(3.0–5.0)
7	7	88a	82.8ab	3.3d	3.0e	2.6f
		(78–100)	(81–84)	(2.4–4.8)	(1.9–4.5)	(1.6–4.1)
8	8	84abc	82.7abc	4.2bc	3.8bcd	3.3b–e
		(77–91)	(81–84)	(3.0–5.0)	(2.6–5.0)	(2.3–5.0)
10	10,19	79bcd	82.2abc	4.1c	3.7cd	3.1c–f
		(59–97)	(80–85)	(3.3–5.0)	(3.0–5.0)	(2.3–5.0)
13	13	81abc	82.7abc	4.5abc	4.2abc	3.7abc
		(79–87)	(82–84)	(3.6–5.0)	(3.5–5.0)	(2.8–4.5)
15	15	77cd	82.4abc	4.8ab	4.5a	4.0a
		(62–84)	(81–84)	(4.0–5.0)	(3.5–5.0)	(2.8–4.5)
16	16	86ab	81.6abc	4.4bc	4.0a–d	3.0def
		(78–95)	(80–83)	(3.9–5.0)	(3.1–4.5)	(2.0–4.0)
20	Control	78cd	81.5cd	4.1c	3.5de	2.7ef
		(69–85)	(80–82)	(3.5–5.0)	(3.0–4.0)	(2.0–3.5)
LSD		8	1.3	0.6	0.6	0.7

[a] Average from two trials of lines planted in three locations (range of values in parentheses). Values followed by the same letter in the same column are not significantly different ($P < 0.05$). LSD = least significant difference ($P < 0.05$).

(2001) reported that deletion of HMW-GS subunits at any *Glu-1* locus reduced dough mixing strength. For group 13, the dough weakening effect of the 2 + 12 allelic pair (Payne et al 1987) may have been more prominent in the presence of both alleles 2* and 1 on the *Glu-A1* locus.

On the other hand, sample groups 6 and 15 had the smallest diameter tortillas (161–163 mm) but were similar to the control (Fig. 1). For group 15, the small diameter was expected based on dough textural properties (Table III). Both groups 6 and 15 contained 2* and 5 + 10 on the *Glu-A1* and *Glu-D1* loci, respectively. Thus, it seemed the dough-strengthening effect of the 1Dx5 + 1Dy10 allelic pair (Payne et al 1987) was accentuated in the presence of a 2* allele on *Glu-A1* and 17 + 18 or 7 + 9 on *Glu-B1*. Generally, based on the protocol used in our laboratory, a tortilla diameter of ≥165 mm is considered acceptable. Somewhat unexpectedly, sample group 10, which had dough with the highest compression force and resistance to extension, as well as one of the least extensible doughs (Table III), produced intermediate-diameter tortillas (mean = 166 mm). This may be related to the presence of the 2 + 12 allelic pair on *Glu-D1*, but more importantly it demonstrates the lack of full understanding of how dough properties influence tortilla quality.

Tortilla Flexibility and Rollability. Retaining flexibility during storage is another key quality parameter of tortillas, as the ability of a tortilla to roll as a wrap without cracking or breaking is a prerequisite for its utilization. Tortilla flexibility will reduce gradually during storage, as shown in Table IV. A flexibility score ≥3.0 after at least 16 days of storage is considered acceptable (Alviola and Waniska 2008). This flexibility score is typically difficult to achieve, because tortillas that maintain their flexibility over time (shelf stability) tend to have unacceptable diameter (too small) and also to have a chewy texture. Thus, the delicate compromise between acceptable diameter and sensory textural attributes as well as shelf stability remains a challenge.

Among the lines tested, only groups 2 and 7 had unacceptable rollability scores (<3.0) at day 16 (Table IV). These sample groups also had among the largest diameter tortillas, besides group 16 (Fig. 1). As previously explained, the HMW-GS allelic profile of group 2 samples (especially *Glu-B1*) was detrimental to the dough strength (Table III) and resulted in large-diameter tortillas (Fig. 1), and it may have contributed to the faster loss of flexibility over time compared with other samples with the 5 + 10 allele pair on *Glu-D1*. For group 7 samples, deletion on *Glu-A1* likely contributed to the poor flexibility score by day 16, which suggested that the HMW-GS expressed by *Glu-A1* alleles were important to tortilla quality.

Sample groups 6 and 15 had the most flexible tortillas at day 16 (Table IV); these samples also had the smallest diameter tortillas among the lines tested (Fig. 1), further illustrating the negative correlation between diameter and flexibility. Thus, even though these samples had near-excellent shelf stability, they would not be ideal for tortillas without the use of reducing agents to weaken the gluten structure. Following closely in flexibility scores were sample groups 1, 5, and 13, which had scores of 3.7–3.8 (Table IV). Group 1 and 5 samples had intermediate dough textural properties and tortilla diameter, whereas group 13 had one of the larger tortilla diameters (Table III, Fig. 1). It was apparent that a combination of either 17 + 18 or 7 + 9 allelic pairs on the *Glu-B1* locus and 5 + 10 on the *Glu-D1* locus produced tortillas with very good shelf stability, but the dough was too elastic (produced small-diameter tortillas) in the presence of allele 2* on *Glu-A1*, as seen for groups 6 and 15. On the other hand, when allele 1 instead of 2* was present on *Glu-A1*, the gluten elasticity was reduced sufficiently to produce acceptable diameter while still maintaining very good shelf stability. Substituting the dough-strengthening 5 + 10 on *Glu-D1* with 2 + 12 also produced a similar effect, as seen for groups 1 and 13, and to some extent, for group 10.

Sample group 16, which produced the largest diameter tortillas, was expected to have very poor shelf stability based on the known negative correlation between diameter and shelf stability (Pascut et al 2004). However, this group produced tortillas that were still acceptable at day 16 based on flexibility score (day 16 score = 3.0). This result is especially exciting and shows important promise of this allelic composition as a tortilla wheat line. Further investigation, especially on how factors like growth environment affect dough-mixing and tortilla-making properties of this HMW-GS allele composition, is warranted. It would also be interesting to establish how other variations of alleles on *Glu-B1* and *Glu-A1* in combination with allele 5 on *Glu-D1* influence the dough rheology and tortilla-making attributes of wheat.

Objective Textural Properties. Large textural differences were noticeable between day 0 and day 4 of storage, with smaller changes between day 8 and day 16, similar to previous findings (Bejosano et al 2005) (Fig. 2). The work to rupture (measured by the two-dimensional extensibility test) integrates the area under the curve of distance versus force to rupture a tortilla disk, and it is a good indicator of tortilla sensory textural quality (Alviola and Awika 2010). The objective tortilla textural properties generally

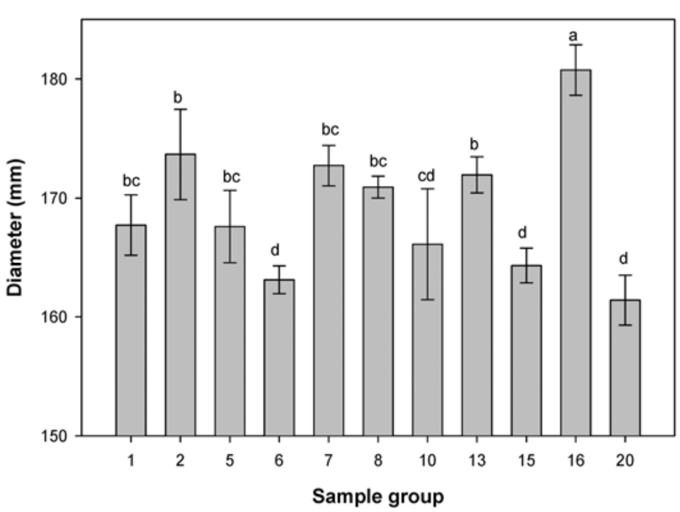

Fig. 1. Effect of variations in high-molecular-weight glutenin allele composition on tortilla diameter. Error bars represent ± standard error of the means for the three environments; bars with the same letter are not significantly different (α = 0.05). Wheat lines are identified in Table I.

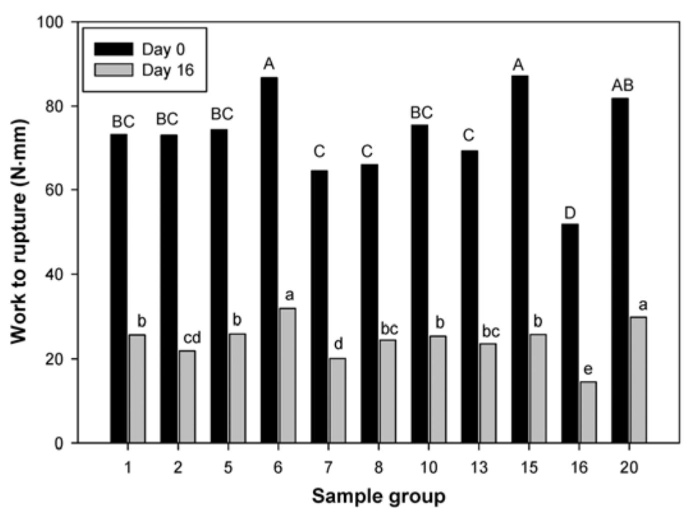

Fig. 2. Effect of variations in high-molecular-weight allele composition on work to rupture tortillas over storage. Bars with similar letters in the same case indicate similar flexibility scores; upper case letters for day 0, lower case for day 16. Wheat lines are identified in Table I.

agreed with dough textural parameters, as well as with other tortilla attributes. For example, the three sample groups that had the lowest tortilla diameter (control, 6, and 15) (Fig. 1) required the highest work to rupture at days 0 and 16, which was an indication of tough (chewy) texture. This result further confirmed that the allele combination expressed by sample groups 6 and 15 produced dough that was too strong and may not be ideal for tortilla making.

On the other hand, sample group 16, which produced the largest tortillas, also required the least work to rupture over storage days (indicating the most tender texture), followed by sample groups 7, 8, and 13, all of which had large-diameter tortillas (>170 mm) (Fig. 1). Tortilla diameter and work to rupture correlated strongly, with $r = 0.92$ and 0.94 for day 0 and day 16, respectively. This correlation illustrates the impact of dough elasticity on tortilla textural attributes that have implication on sensory quality. With the exception of group 7 samples, the tortilla groups that required lower force to rupture also had acceptable shelf stability and are thus promising as identity-preserved lines for potential tortilla production.

Perspective on Important Findings. Some interesting overall patterns were observed in relation to HMW-GS allelic composition and tortilla quality. The lines that produced the "best" tortillas (diameter above 170 mm, rollability score above 3.5 at day 16) had 2*, 17 + 18, and 2 + 12 at *Glu-A1, B1,* and *D1*, respectively (lines 1 and 13; Table IV, Fig. 1). The other lines with similar composition at *Glu-A1* and *D1* but a different allelic composition at *Glu-B1* (lines 8 and 10) also produced acceptable quality tortillas (diameter above 165 mm, rollability score above 3.0 at day 16). Thus, all wheat groups encoding for 2 + 12 at *Glu-D1* produced good quality tortillas. Presence of 2 + 12 at *Glu-D1* has been reported to weaken dough strength relative to 5 + 10 at the same domain (Lukow et al 1989; Wang et al 1993). In fact, Lukow et al (1989) reported that almost all wheat lines encoding 2 + 12 at *Glu-D1* had poor breadmaking quality and that subunit 5 + 10 was needed at this locus to provide adequate gluten strength for bread. Wang et al (1993) observed that within *Glu-1*, composition at subunits *A1* and *D1* accounted for the majority (≈86%) of variations in gluten strength as measured by a microsedimentation test. In our study, it was clear that the *Glu-D1* allele encoding subunits 2 + 12 was a major contributor to desirable tortilla quality. This result confirmed that functionality of wheat proteins essential for bread quality will likely not produce good tortillas and vice versa. The influence of *Glu-A1* alleles on *Glu-D1* 2 + 12 functionality could not be ascertained in this study because all of the lines encoding subunits 2 + 12 at *Glu-D1* also encoded 2* at *Glu-A1*. On the other hand, at the *Glu-B1* domain, 17 + 18 subunits (compared with 7 or 7 + 9) enhanced tortilla-making quality of lines encoding 2 + 12 at *Glu-D1*.

As expected, the lines that produced the most elastic doughs encoded 5 + 10 at *Glu-D1*; these lines also tended to have higher IPP content (Tables II and III). Consequently, the smallest diameter tortillas were produced by lines encoding subunits 5 + 10 at *Glu-D1* (Fig. 1). However, among the lines encoding 5 + 10 at *Glu-D1*, the relative influence of *Glu-A1* and *Glu-B1* was more apparent than was observed for lines encoding 2 + 12 at *Glu-D1*. For example, in the presence of 5 + 10 at *Glu-D1*, 17 + 18 at *Glu-B1* weakened the dough enough to produce tortillas with acceptable diameter and quality, but only when *Glu-A1* encoded subunit 1. When 2* was present instead at *Glu-A1,* the dough was too strong, whereas when *Glu-A1* was null, the dough was too weak for tortillas (Tables III and IV, Fig. 1). Such apparent epistatic and additive effects for wheat lines encoding 5 + 10 at *Glu-D1* were previously observed (Kolster et al 1991); the authors also observed that the relative dough-strengthening effect of *Glu-D1* subunits 5 + 10 compared with 2 + 12 increased as the protein content of wheat flour increased. In this case, the protein contents were within a very narrow range and likely did not contribute significantly to the observed differences.

Thus, it seems that wheat lines expressing 2 + 12 alleles at the *Glu-D1* locus provide a more likely viable target for tortilla production than the lines encoding for 5 + 10 at *Glu-D1,* which are more suitable for bread. With the relatively high frequency of occurrence of the 2 + 12 subunits at *Glu-D1* reported in the world wheat collection (Lukow et al 1989; Morgunov et al 1990; Kolster et al 1991; Wang et al 1993), it is likely that development of specialty wheat lines targeting tortillas (and possibly other flatbreads) adaptable to various regions is possible. However, in this study we did not identify a definitive wheat protein compositional attribute that was a good predictor of tortilla quality. This likely suggests that other parameters, such as starch properties and water-soluble polysaccharides, may exert significant influence on tortilla properties and should be investigated. For example, we recently observed that a commercial high-protein bread flour that produced tortillas with very good shelf stability compared with commercial tortilla flour also contained starch that had a lower setback viscosity (as measured by the Rapid Visco Analyzer) than the commercial tortilla flour (Alviola et al 2012). Setback viscosity is related to amylopectin retrogradation; starch retrogradation likely contributes significantly to tortilla staling and loss of rollability.

CONCLUSIONS

It is apparent from this study that the allelic variations in the *Glu-1* loci of wheat can be manipulated to produce a combination of flour protein profile and dough-making attributes that provide desired functionality. The data indicate a promising possibility to produce wheat with optimum functionality for tortillas. Tortillas from wheat lines encoding subunits 2 + 12 at *Glu-D1* had large diameter and acceptable flexibility scores (≥3.0) after 16 days of storage. The wheat lines encoding 2 + 12 at *Glu-D1* are good candidates for development of wheats optimized for tortilla production. Additionally, the wheat lacking HMW-GS at *Glu-D1* was particularly interesting in terms of its unusually extensible dough, which produced very large diameter tortillas while still retaining acceptable shelf stability after 16 days of storage. The deletion at *Glu-D1* may present an additional interesting opportunity to develop identity-preserved tortilla wheat lines.

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